

## Novel Chromosome-Encoded CTX-M-78 $\beta$ -Lactamase from a *Kluyvera georgiana* Clinical Isolate as a Putative Origin of CTX-M-25 Subgroup<sup>▽</sup>

While TEM- and SHV-derived extended-spectrum  $\beta$ -lactamases (ESBLs) arose by point mutations in genes encoding broad-spectrum variants that were extensively disseminated into mobile elements, CTX-M  $\beta$ -lactamases are derived from natural (chromosomal) counterparts that already have oxyimino-cephalosporinase activity (11). To date, the CTX-M/KLU  $\beta$ -lactamases account for nearly 110 representatives clustered in five main groups (<http://www.lahey.org/studies/webt.asp>): CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. A hypothetical chromosomal counterpart for the CTX-M-25 subfamily is still missing.

*Kluyvera georgiana* 14751 was isolated from a bloodstream infection in a 62-year-old male patient through the SENTRY Antimicrobial Surveillance Program in Louisville, KY, on 2 December 2002. Biochemical classification, ambiguous between *Kluyvera georgiana* and *Kluyvera ascorbata*, was resolved by 16S rRNA gene sequencing (GenBank accession no. AM933755), displaying 99.7% identity with the sole available sequence from *K. georgiana* ATCC 51603 (GenBank accession no. AF047186). Identity to all deposited *K. ascorbata* sequences ranged from 97.4 to 98.5% (6).

The strain was resistant to ampicillin, gentamicin, nalidixic acid (Table 1) and trimethoprim-sulfamethoxazole (TMP-SMX) (by disc diffusion). Conjugation to *Escherichia coli* CAG12177 resulted in *E. coli* CK10 harboring an IncFII group conjugative plasmid of ca. 30 kb (pTC10; partial sequence deposited in EMBL under accession no. FN568351), responsible for TMP-SMX and aminoglycoside resistance (Table 1) by a class 1 integron harboring *dfrA17* and *aadA5* gene cassettes (previously reported individually in different *Kluyvera intermedia* isolates; EMBL accession no. EU523051 and EU523052). Upstream of the integron we found a *bla*<sub>TEM-1b</sub> gene followed by a region, including the *Tn3-mpR*, the first 91 bp of *Tn3-mpA*, and an IS26 which is also found in plasmids carrying some *bla*<sub>CTX-M</sub> genes. However, no CTX-M could be recovered from the transferred plasmid.

Partially EcoRI-digested chromosomal DNA from *K. georgiana* 14751 was cloned in a pK19 vector (Kan<sup>r</sup>) and transformed into *E. coli* Top10F' (*E. coli* TKE14751-1KA2), yielding pTKE-1KA2 with an 8-kb insert (Fig. 1) harboring a *bla* gene (876 bp) which encodes a novel CTX-M-78 (EMBL accession no. AM982522) closely related to CTX-M-39 (96.2% amino acid identity) (1) and clustered in the CTX-M-25 subgroup. As seen in Table 1, the MIC increases for cefotaxime, while that of ceftazidime is almost unaffected, correlating with the expected (and experimental [data not shown]) kinetics for most CTX-M enzymes.

As in other chromosome-encoded *bla*<sub>CTX-M/KLU</sub>, a 1,227-bp *att*-like gene encoding a putative aspartate aminotransferase is located upstream, with at least 96% identity with other entries from *Kluyvera*. Downstream, an *orf3*-like gene similar to a putative *ttrR* response regulator from *Kluyvera georgiana* (5) and similar to the *orf3* version from the complex class 1 integrons associated with *bla*<sub>CTX-M-2</sub> (found as a fusion gene *orf3::qacEΔ1*) (7), and an *orf4*-like gene encoding a putative autotransporter in *Kluyvera ascorbata* (2), were also found. In

TABLE 1. MICs of antibiotics for *Kluyvera georgiana* 14751, the recipient strain, and the derived recombinant clone

Antibiotic(s)	MIC (μg/ml) <sup>a</sup>		
	<i>K. georgiana</i> 14751	<i>E. coli</i> TKE14751-1KA2	<i>E. coli</i> Top10F'
Ampicillin	256	256	2
Ampicillin-clavulanate	4/2	2/1	2/1
Cephalothin	8	256	2
Cefoxitin	1	2	2
Cefotaxime	0.032	1	≤0.016
Ceftazidime	0.063	0.25	0.125
Cefepime	0.032	0.5	0.016
Aztreonam	≤0.016	0.5	0.032
Kanamycin	16	256	1
Gentamicin	64	≤0.5	ND
Tetracycline	≤0.5	256	ND
Nalidixic acid	128	2	ND

<sup>a</sup> For ampicillin-clavulanate, the MIC values for ampicillin and clavulanate are shown before and after the slash, respectively. ND, not determined.

contrast to other *kluyveras*, in which sequences similar to IRr-ISEcp1 were found downstream of *bla*<sub>CTX-M/KLU</sub> (8), we did not observe any sequence that could serve as putative right inverted repeats (IRrs) there (Fig. 1). A 35-bp sequence seems to represent the boundary between the chromosome-derived information and the plasmid-acquired information. In *bla*<sub>CTX-M-25</sub> and *bla*<sub>CTX-M-26</sub> (GenBank accession no. AF518567 and AY455830, respectively) (4), the immediate upstream region is occupied by ISEcp1, also associated with other *bla*<sub>CTX-M</sub> genes (3). A putative 5-bp target site for ISEcp1B (AATAC) was found immediately upstream of the 35-bp sequence in the chromosomal DNA from *K. georgiana* 14751.

CTX-M-78 possesses high similarity with members of the CTX-M-25 subgroup, making this enzyme the closest representative to be considered one of the probable progenitors of the subfamily.

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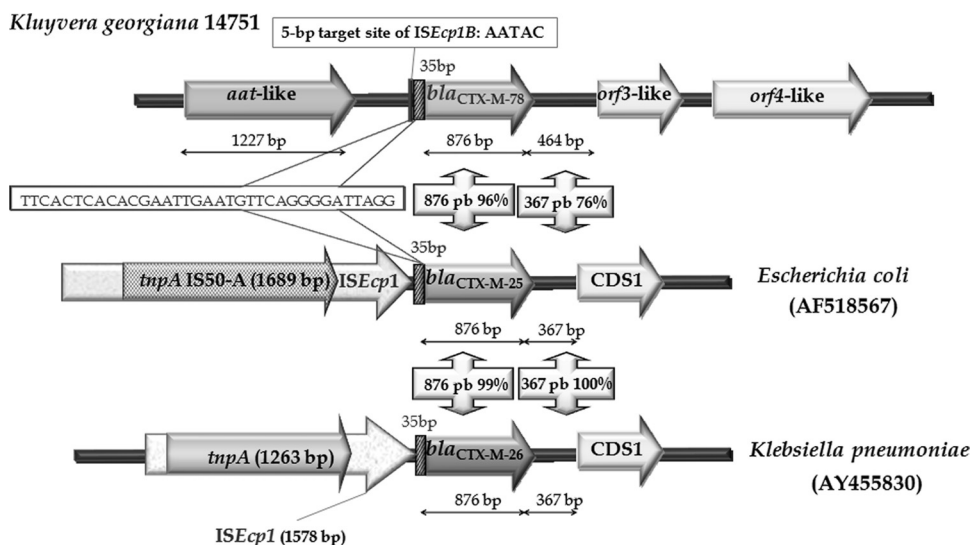


FIG. 1. Schematic representation of the insert of pTKE-1KA2 from *Kluyvera georgiana* 14751 (top) and comparison with available sequences harboring *bla*<sub>CTX-M-25</sub> (middle) and *bla*<sub>CTX-M-26</sub> (bottom).

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